

A preliminary analysis of phylogenetic relationships of *Arundinaria* and related genera based on nucleotide sequences of nrDNA (ITS region) and cpDNA (*trnL-F* intergenic spacer)

ZHUGE Qiang¹, DING Yu-long², XU Chen¹, ZOU Hui-yu¹, HUANG Min-ren¹, WANG Ming-xiu^{1*}

1. Laboratory of Forest Genetics and Gene Engineering, Nanjing Forestry University, Nanjing 210037, P. R. China

2. Center of Bamboo Research, Nanjing Forestry University, Nanjing 210037, P. R. China

Abstract: Phylogenetic relationships of *Arundinaria* and related genera (*Pleioblastus*, *Pseudosasa*, *Oligostachyum*, *Bashania*, *Clavinodum*, etc.) were assessed by analyzing the sequences of the nrDNA internal transcribed spacer (ITS) and the cpDNA *trnL-F* intergenic spacer (IGS). Comparison with *trnL-F* IGS sequence, the ITS region provided the higher number of parsimony informative characters, and the interspecific variation of the ITS sequence was higher than that of the *trnL-F* IGS sequence. The tree obtained by combining both sets of data showed that the species sampled in *Arundinaria* and the related genera were monophyletic and divided into two clades. The relationships and positioning of all the taxa surveyed (including *A. oleosa*, *A. hsienchuensis*, *A. chino*, *A. amara*, *A. yixingensis*, *A. amabilis*, *A. fortunei*, *A. pygmaea*, *A. gramineus*, *A. fargesii*, *A. faberi*, *A. hupehense*, *Pseudosasa japonica* cv. *Tsutsumiana*, *P. japonica* and *Brachystachyum densiflorum*) were also discussed. The results from the sequences were broadly consistent with morphological characters, appearing all these taxa sampled belong to the genus of *Arundinaria*. The topologies of the trees generated from individual data and the combined data were similar.

Keywords: *Arundinaria*; Internal transcribed spacers (ITS) sequences; *trnL-F* intergenic spacer (IGS) sequences; Phylogenetic relationships
CIC number: Q344.1 **Document Code:** A **Article ID:** 1007-662X(2005)01-0005-04

Introduction

Arundinaria is the earliest genus to be established in the genera of monopodial bamboos with the most diverse bamboos species in the world. It is reported that there were about 400 species under *Arundinaria*. However, up to now, there is no comprehensive system of classification which indicates species relationships within the bamboos in the *Arundinaria* and the related genera (including *Pleioblastus*, *Pseudosasa*, *Oligostachyum*, *Bashania*, *Clavinodum*, etc.) due to systematic difficulties of the availability of good taxonomic characters (Guo & Li 2002). The systematical classification of *Arundinaria* is still controversial, with taxa which have been alternatively merged into the genus of *Arundinaria*, or segregated into several related genera (e.g. *Pleioblastus*, *Pseudosasa*, *Oligostachyum*, *Bashania*, *Clavinodum*, etc.). Different views on the status of these genera have been made by Yang *et al.* (1993), Soderstrom (1987), Li *et al.* (2002), etc.

Molecular data from DNA studies have been widely applied in genetic variation, systematical classification, and phylogenetic relationship of bamboos, which mainly based on randomly amplified polymorphic DNA (RAPD) (Ding 1998), simple sequence repeat (SSR) (Li *et al.* 2002), and restriction fragment length polymorphism (RFLP) (Friar & Kochert 1994), etc. The studies based on two different regions of the bamboo genome, ITS sequences from nrDNA and *trnL-F* sequences from cpDNA,

have been scarcely reported before. The objectives of the this study are: (1) to evaluate the level at which molecular data from these two regions provide phylogenetic information, and (2) to test if the combination of the ITS region and the *trnL-F* intergenic spacer (IGS) sequences could provides valuable data for phylogenetic relationship within the species traditionally described to the *Arundinaria* and the related genera (including *Pleioblastus*, *Pseudosasa*, *Oligostachyum*, *Bashania*, *Clavinodum*, etc.).

Materials and methods

Seventeen species representing related genera of *Arundinaria* (*Arundinaria gramineus* is the model species of *Pleioblastus*, *Arundinaria fargesii* the model species of *Bashania*, *Arundinaria sulcata* the model species of *Oligostachyum*, *Pseudosasa japonica* the model species of *Pseudosasa*, *Arundinaria oedogonata* is the model species of *Clavinodum*) and one outgroup (*Phyllostachys edulis*) were sampled (Table 1).

Total DNA was extracted from fresh leaves by the DNeasy Plant Mini Kit (QIAGEN). Nuclear ribosomal internal transcribed spacers (ITS1, 5.8S, ITS2 region) were amplified using the forward primer (ITS-1 at 18S: 5'-AGAAGTCGTAACAAGGTTTCCGTAGG-3') and the reverse primer (ITS-4 at 26S: 5'-TCCTCCGCTTATTGATATGC-3'), the *trnL-F* intergenic spacer of the chloroplast genome was amplified using the primers e and f (Taberlet *et al.* 1991). The PCR products were purified by Wizard PCR Preps DNA Purification System (Promega), and then sequenced directly on ABI 310 DNA automated sequencer using ABI Prism Bigdye™ Terminator Cycle Sequencing Beady Reaction Kit.

The entire ITS and *trnL-F* sequences of 18 taxa were aligned with the CLUSTAL W multiple sequence alignment program (Thomson *et al.* 1997), and were adjusted manually in the

Foundation item: This study was supported by National Natural Science Foundation of China (No. 30170788)

Biography: ZHUGE Qiang (1959-), male, professor in Laboratory of Forest Genetics and Gene Engineering, Nanjing Forestry University, Nanjing 210037, P. R. China. E-mail: qzhuge@njfu.edu.cn.

Received date: 2004-10-11

Responsible editor: Song Funan

*Corresponding author

sequences where necessary. Phylogenetic relationships were analysed by PAUP 4.0 (Swofford 1998) with all changes weighted equally, using heuristic search, random addition sequence, and tree bisection-reconnection swapping. Gaps were

treated as missing data. Searches were conducted on the ITS data, on the *trnL*-F data and on the ITS and the *trnL*-F combined data. Bootstrap analyses of 1 000 replications were performed to show relative supports for individual clades.

Table 1. Materials of 18 species of the bamboos sampled in this report

Taxa	Sources	Collector	Voucher ^①
<i>Arundinaria oleosa</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ2—103
<i>Arundinaria amara</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ15—03
<i>Arundinaria hsienchuensis</i>	Hangzhou Bamboo Garden	ZHUGE Qiang	HZS—12
<i>Arundinaria yixingensis</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ15—07
<i>Arundinaria gramineus</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ4—36
<i>Arundinaria chino</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ15—10
<i>Pseudosasa japonica</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ16—03
<i>Pseudosasa japonica</i> cv. <i>Tsutsumiana</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ9—15
<i>Arundinaria amabilis</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ6—15
<i>Arundinaria fortunei</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ5—05
<i>Arundinaria pygmaea</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ8—10
<i>Arundinaria sulcata</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ3—06
<i>Arundinaria hupehense</i>	Hangzhou Bamboo Garden	ZHUGE Qiang	HZ4a—01
<i>Arundinaria fargesii</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ3—20
<i>Arundinaria oedogonata</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ8—06
<i>Arundinaria faberi</i>	Hangzhou Bamboo Garden	ZHUGE Qiang	HZK—5
<i>Brachystachyum densiflorum</i>	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ13—04
<i>Phyllostachys edulis</i> (outgroup)	Bamboo Garden of Nanjing Forestry University	DING Yu-Long	NJ21—03

Note: ①The vouchers were stored at the herbarium of Nanjing Forestry University

Results and discussion

The length of the entire ITS region (including 5.8S) in all taxa sampled ranged from 596 to 623 bp, with ITS1 ranging from 215 to 225 bp, 5.8S 163 bp for all species, and ITS2 ranging from 213 to 231 bp, and the (G+C) contents in ITS2 (ranged from 72.3% to 76.9%) are slightly higher than those in ITS1 (ranged from 69.6% to 74.5%). In the data matrix, the levels of potentially informative nucleotide site (14.3%) of ITS1 are somewhat higher than those of ITS2 (6.7%). The similar pattern was also found in *Krigia* (Kim & Jansen 1994), *Populus* (Shi *et al.* 2001). The aligned *trnL*-F data matrix has 116 variable characters and 15 (1.5%) potentially informative characters out of 973 characters. The sequences are, typically for chloroplast DNA, rich in AT (66.6%). Of the 1 658 aligned ITS and *trnL*-F combined sequences, 1 405 (84.7%) are constant, 186 (11.2%) uninformative and 67 (4.1%) are informative (Table 2).

Heuristic searches including only ITS characters generated three most parsimonious trees requiring 205 evolutionary steps (strict consensus in Fig. 1) with a consistency index (CI) (0.8293) and retention index (RI) (0.7083). The ITS data indicates that the species sampled in *Arundinaria* and the related genera are monophyletic and divided into two clades with stronger support (bootstrap values >50%). One clade included *A. oleosa*, *A. hsienchuensis*, *A. chino*, *A. amara*, *A. yixingensis*, *A. amabilis*, *A. fortunei* and *A. pygmaea*, and the other contained *A. gramineus*, *A. fargesii*, *A. faberi*, *A. hupehense*, *Pseudosasa japonica* cv. *Tsutsumiana*, *P. japonica*, *Brachystachyum densiflorum*, *A. oedogonata* and *A. sulcata*. Therefore, it is likely that all these taxa sampled belong to *Arundinaria*, which should include the related genera (*Pleioblastus*, *Pseudosasa*, *Oligostachyum*, *Bashania*, *Clavinodum*). The result also shows that there is a close relationship between *A. gramineus* and *A. fargesii*,

Pseudosasa japonica cv. *Tsutsumiana* and *P. japonica*, *A. sulcata*, *Brachystachyum densiflorum* and *A. oedogonata* (99%, 100% and 87% bootstrap support, respectively). Furthermore, it is shown from the phylogenetic tree that *A. pygmaea* and *A. fortunei* have a close relationship, and are a sister branch to *Pleioblastus* bamboos.

Table 2. ITS region and *trnL*-F intergenic spacer (IGS) sequences characteristics of the bamboos sampled

Parameter	ITS	<i>trnL</i> -F	combined
Number of taxa	18	18	18
Sequence length range (bp)	596-623	868-963	1467-1560
Total aligned matrix length (bp)	669	973	1658
G+C content (%)	69.7	33.4	48.1
Number of constant sites, %	519 (77.6%)	842 (86.5%)	1405 (84.7%)
Number of variable sites, %	96 (14.3%)	116 (11.9%)	186 (11.2%)
Number of uninformative sites, %	42 (91.9%)	101 (98.5%)	119 (95.9%)
Number of informative sites, %	54 (8.1%)	15 (1.5%)	67 (4.1%)

The phylogenetic analysis by *trnL*-F IGS region sequences produce one most parsimonious tree (Fig. 2) of 139 steps length, with consistency index (CI) (0.9495) and retention index (RI) (0.7500). The topology of the tree of the *trnL*-F dataset is similar to the ITS tree, and also shows a monophyly of the species sampled in *Arundinaria* and the related genera which are fell into two clades. One clade includes the same species with similar relationships as in the ITS tree, except that the position of *A. amara* and *A. yixingensis* are replaced by *A. gramineus*, *A. sulcata*, *P. japonica* cv. *Tsutsumiana* and *P. japonica*.

The maximum parsimony analyses of the combined data of ITS region and *trnL*-F IGS region generate one most parsimonious tree (Fig. 3) of 332 steps length, with a consistency index (CI) (0.8404), a retention index (RI) (0.6936) and rescaled consistency index (RC) (0.5829). The phylogenetic tree from

both sets of data is broadly similar in the topologies of the trees from the individual data, showing a monophyly of the species sampled in *Arundinaria* and the related genera (including *Pleiolobatus*, *Pseudosasa*, *Oligostachyum*, *Bashania*, *Clavinodum*, etc.) which are divided into two clades. One clade

comprises *Pseudosasa japonica* cv. *Tsutsumiana*, *P. japonica*, *A. yixingensis*, *A. amara*, *A. oleosa*, *A. hsienchuensis*, *A. amabilis*, *A. chino*, *A. fortunei* and *A. pygmaea*, and the other comprises *Brachystachyum densiflorum*, *A. oedogonata*, *A. sulcata*, *A. hupehense*, *A. fargesii*, *A. gramineus* and *A. faberi*.

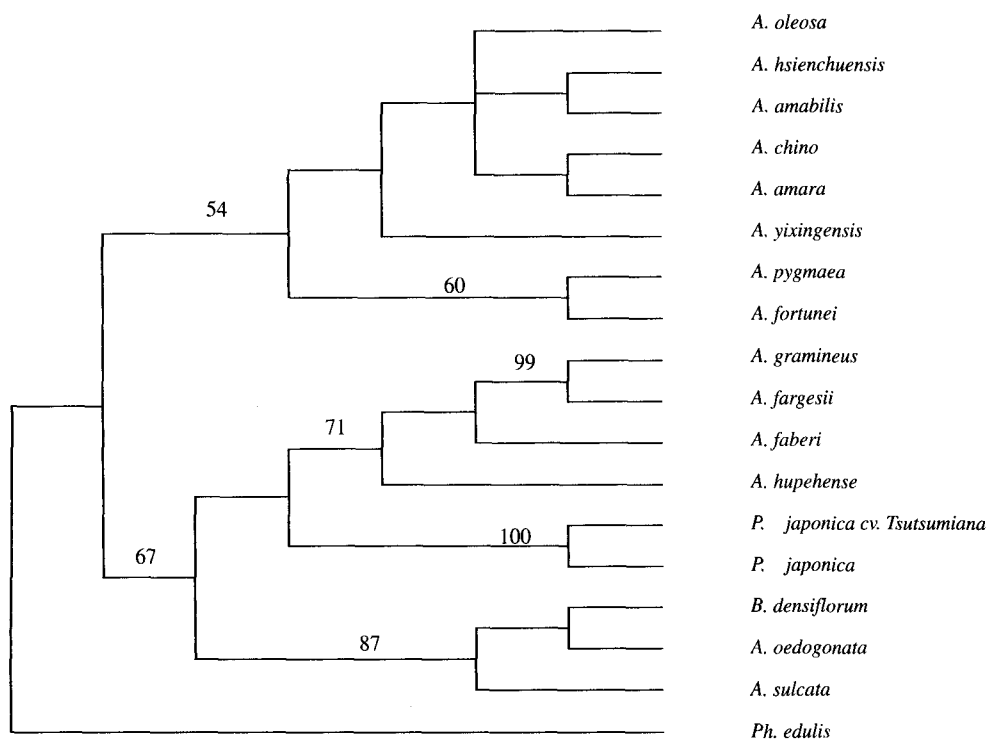


Fig. 1 Strict consensus tree of the 3 most parsimonious trees from the entire ITS sequences with gaps coded as missing. Tree length, 205 steps; CI, 0.8293; RI, 0.7083. Numbers above lines represent bootstrap values in 1 000 replicates.

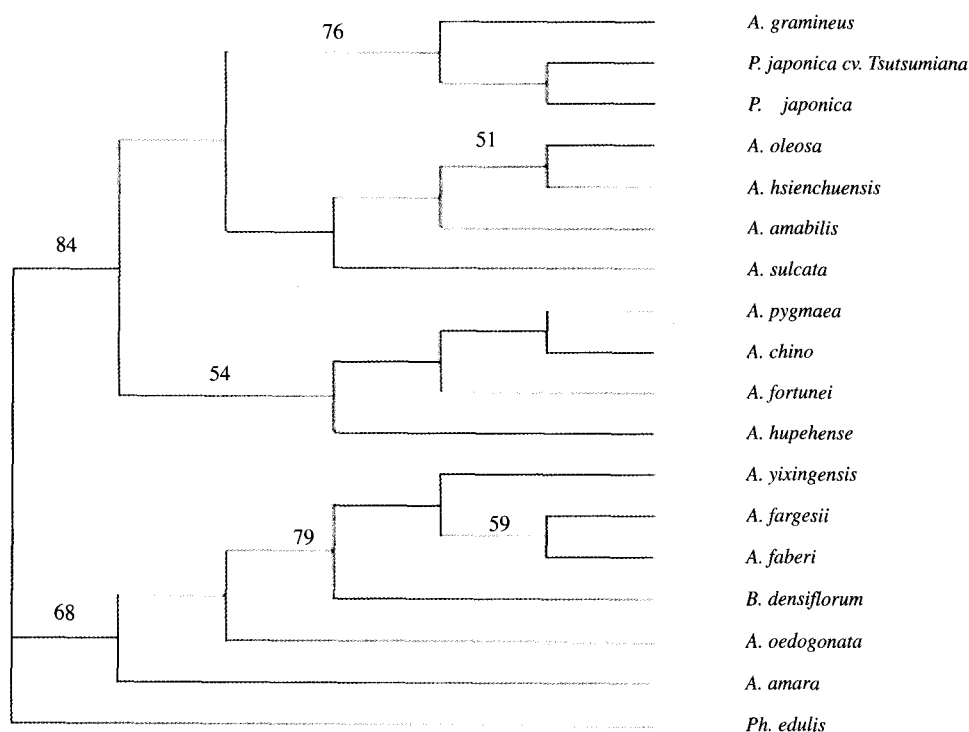


Fig. 2 The most parsimonious tree from the *trnL-F* IGS region sequences with gaps coded as missing. Tree length, 139 steps; CI, 0.9495; RI, 0.7500. Numbers above lines represent bootstrap values in 1 000 replicates.

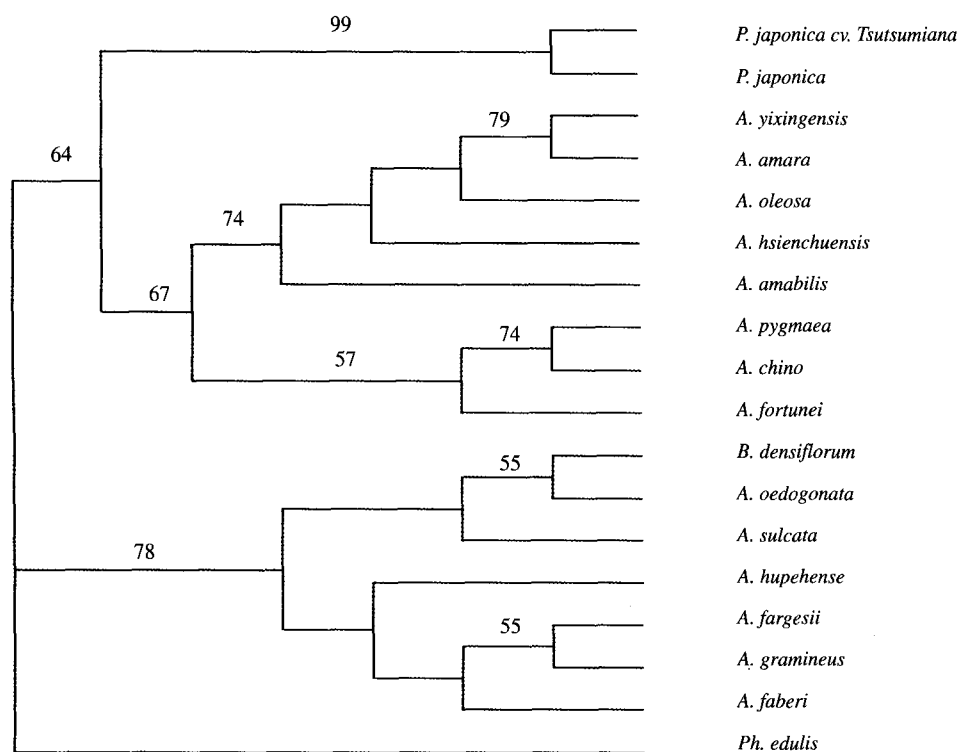


Fig. 3 The most parsimonious tree from the combined data of ITS region and trnL-F IGS region sequences with gaps coded as missing. Tree length, 332 steps; CI, 0.8404; RI, 0.6936. Numbers above lines represent bootstrap values in 1 000 replicates.

Our study indicated that the two regions of ITS and *trnL-F* IGS provide an important clue for the establishment of phylogenetic relationships of the bamboos. The ITS sequence is not only alignable at intergeneric level in *Arundinaria*, but it is also phylogenetically useful at this level. Comparison with *trnL-F* IGS sequence, the ITS region provides the higher number of parsimony informative characters, and the interspecific variation of the ITS sequence is higher than that of the *trnL-F* IGS sequence. The topologies of the trees generate from individual data and the combined data are similar. The results are significant in confirming aspects of previous morphological analyses (Yang & Zhao 1993).

Acknowledgements

We are grateful to Professor ZHAO Qi-sen of Nanjing Forestry University and Professor YANG Guang-yao of Jiangxi Agriculture University for helpful comments on the manuscript.

References

- Ding, Y.L. 1998. Systematic studies on *Phyllostachys* [D]. Dissertation for Ph.D. Nanjing: Nanjing Forestry University. (in Chinese)
- Friar, E., Kochert, G. 1994. A study of genetic variation and evolution of *Phyllostachys* (Bambusoideae: Poaceae) using nuclear restriction fragment length polymorphisms [J]. *Theoretical and Applied Genetics*, **89**: 265–270.
- Guo, Z.H., Li, D.Z. 2002. Advances in the systematics and biogeography of the Bambusoideae (Gramineae) with remarks on some remaining problems [J]. *Acta Botanica Yunnanica*, **24**(4): 431–438. (in Chinese)
- Kim, K.J., Jansen, R.K. 1994. Comparison of phylogenetic hypothesis among different data sets in dwarf dandelions (*Krigia*): Additional information from internal transcribed spacer sequences of Nuclear ribosomal DNA [J]. *Plant Syst Evol.*, **177**: 157–185.
- Li, S.X., Yin, T.M., Zou, H.Y., Ding, Y.L., Huang, M.R. 2002. Preliminary study on molecular systematics of bamboo by SSR primers derived from rice [J]. *SCIENTIA SILVAE SINICAE*, **38**(3): 42–48. (in Chinese)
- Shi, Q.L., Zhuge, Q., Huang, M.R., Wang, M.X. 2001. Phylogenetic relationship of *Populus* sections by ITS sequence analysis [J]. *Acta Botanica Sinica*, **43**(3): 323–325. (in Chinese)
- Soderstrom, T.R., Ellis, R. 1987. The position of bamboo genera and allies in a system of grass classification. In: Soderstrom T R, Hilu K W, Campbell C S, *et al.* (eds.), *Grass Systematic and Evolution* [C]. Washington, D.C.: Smithsonian Institution Press.
- Swofford, D.L. 1998. PAUP* 4.0 Phylogenetic analysis using parsimony (and other methods). Sunderland: Sinauer associates.
- Taberlet, P., Gielly, L., Pautou, G., Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA [J]. *Plant Mol. Biol.*, **17**: 1105–1109.
- Thomson, J.D., Gibson, T.J., Plewniak, F. 1997. The clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools [J]. *Nucl Acids Res*, **25**: 4876–4882.
- Yang, G.Y., Zhao, Q.S. 1993. Study on *Arundinaria* in China [J]. *Journal of Bamboo Research*, **12**(4): 1–7. (in Chinese)